An Inexpensive Solid-State Photometer Circuit Useful in Studying Bioluminescence

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Bioluminescence has been the subject of much research within the past two decades. Measurement of this phenomenon has usually been by a photomultiplier photometer originally designed by MacNichols in 1952[1] and subsequently modified by Mitchell and Hastings,[2] Strickland,[3] and others. There are many commercially designed photometers that are adequate but unfortunately are quite expensive. Assembly of a suitable instrument from commerically available parts is also expensive and time-consuming, requiring a considerable degree of technical ability.

Recently, inexpensive integrated photodetection assemblies (IPA) have become available from the Radio Corporation of America. These assemblies, complete with an RCA type 931B photomultiplier tube and integral solid-state high-voltage power supply, voltage regulator, and a resistor divider network, are mounted behind an electrostatic shield (Fig. 1). Additionally, low-cost solid-state operational amplifiers can be used to convert the current output of the IPA to voltage and to interface with conventional recording instrumentation and in integration. Finally, inexpensive solid-state power supplies, adequate to drive both the IPA and the operational amplifiers, are available.

As supplied, the IPA requires a 12V or 15V dc power supply and generates the high voltage required to operate the phototube internally. Control of the tube sensitivity or gain is attained by inserting a variable resistor R1 between ground of the external power supply and the internal voltage regulator of the IPA (Fig. 2).

Precise and reproducible settings of the gain control are obtained by means of a turns counting dial attached to the variable resistor R1.

The output from the IPA is connected to the negative input terminal of the current to voltage converter A and inverts the polarity of the signal. The signal is then led to B, an operational amplifier wired as an integrating circuit.

For this circuit:

$$V_0 = -\frac{1}{RC}\int V_A dt$$

where V_A is the output of the current to voltage converter. R and C were chosen to be 1 Mohm and 10 μF to produce a time constant of 10 s which was appropriate to our studies.

Provision for output offset null adjustment is provided by a nulling potentiometer R7 whose function is to zero the output and eliminate drift in the recording instrumentation. Proper biasing of the circuit is attained by adding a fixed and a variable resistor in the noninverting input circuit (R5, R6). Measurement of light intensity without integration is provided by opening S3, and recording at output 1. Light intensity with integration is obtained by closing S3, thus returning C1 to the integration feedback circuit and recording at output 2. A shorting switch, S2, is inserted in the circuit to reset the integrator to zero before each determination.

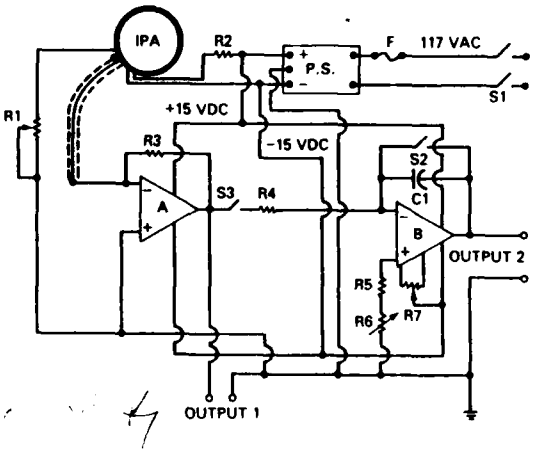

Figure 1. Schematic diagram of components. R1: 5-Kohm, 250-mW variable resistor linear tape 10 turns. R2: 5 ohm. R3: 20 Kohm. R4: 1 Mohm. R5: 0.25 Mohm. R6: 0.75 Mohm. R7: 5 Kohm variable. C1: 10 μF, 50 V dc. Amplifier A: type 741 operational amplifier. Amplifier B: type 741 operational amplifier. P.S.: power supply, 15 V dc, 200 mA, Semiconductor Circuits Inc., Windham, NH 03087, Catalog No. CM 15D200. IPA: integrated photometer assembly, Phototube Marketing, RCA, Lancaster, PA 17604, Catalog No. PF1006A. S1: switch, single-pole, double-throw. S2: grounding switch, single-pole, single-throw. S3: single-pole, single-throw. F: small 3-8 A, 250-V slow burn fuse, Littlefuse U.S.A.

The integrator mode of operation is especially useful in situations where total light output of a source which varies in intensity at a rate faster than the recorder can easily follow is to be measured. Such a situation obtains in the determination of the bioluminescence of organisms whose emission is in the form of a train of flashes.

The circuitry is assembled on a paper phenolic board using commercially available components and housed in a small metal box. No special wiring precautions were observed.

Calibration measurements were made with a Fluke digital multimeter Model 8002A. A hermetically sealed light source containing a phosphor screen excited by krypton-85 gas was used to provide a standard for evaluating instrumentation drift and linearity of response on a periodic basis. Instrumentation drift was checked by regular observations for a period of 6 h using the standard light source and various gain settings. No significant drift was observed.

Linearity of response was determined by interposing various neutral density filters between the standard light source and phototube, and integrating the response over a two-minute period. The slope of the curve was calculated for each neutral density filter at two gain settings. The results are depicted in Figure 3.

[1] Chase, A. M., *Methods Biochem. Anal.*, **8**, 61 (1960).
[2] Mitchell, G. W., and Hastings, J. W., *Anal. Biochem.*, **39**, 243 (1971).
[3] Strickland, J. D. H., *in* "A Practical Handbook of Sea Water Analysis," 2nd ed., Bull. 167, Fisheries Research Board of Canada, Ottawa.

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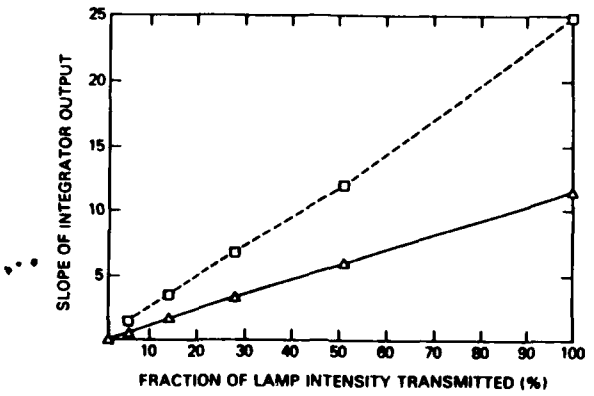

Figure 2. Integrator response as a function of light intensity.

The system described was calibrated with a light source consisting of a standard lamp AJ2239, from RCA Electro Optics and Devices, Lancaster, PA and operated at its rated current at color temperature of 2856°K and a filter set whose absorption spectrum was measured. This combination delivered 7×10^{-5} lux at 35 m, with half band width 60 nm and maximum intensity at 490 nm. The response was 1.8×10^{8} mV/lux at high gain, and 1.3×10^{7} mV/lux at low gain. At high gain, a noise level of $\pm 2 \times 10^{-9}$ lux, corresponding to $\pm 1/4$ mV was detected with a recorder having 0.5 s response time.

The 18 μs rise time of the photometer circuit was measured using a light-emitting diode with nanosecond rise time, and oscilloscope.

A photometer is an essential instrument in analytical procedures involving the measurement of low light level emission.

The instrument we have designed and constructed has been in satisfactory use for several months in our studies of luminescent dinoflagellates. Measurements of the total illumination of as few as 10 cells/ml were easily obtained and were reproducible. The total cost of all components was less than $300.

We feel this instrument will prove useful in bioluminescence measurements such as those using the firefly or bacterial luciferin-luciferase systems and chemiluminescence and fluorescence systems, among other applications.

Acknowledgment

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